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Understanding multiple stressors in a Mediterranean basin: Combined effects of land use, water scarcity and nutrient enrichment

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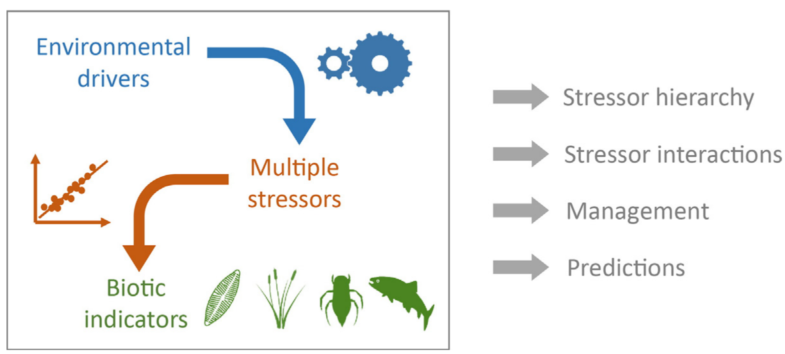
HIGHLIGHTS

- The interplay of water scarcity and nutrients in river biotic state is addressed.
- Stressors were simulated through process-based modelling.
- Stressors, land use and environmental background were used to model biotic state.
- Agriculture and nutrient enrichment showed major effects on biotic state.
- Interactions should be carefully examined to avoid wrong conclusions for management.

GRAPHICAL ABSTRACT



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ABSTRACT

River basins are extremely complex hierarchical and directional systems that are affected by a multitude of interacting stressors. This complexity hampers effective management and conservation planning to be effectively implemented, especially under climate change. The objective of this work is to provide a wide scale approach to basin management by interpreting the effect of isolated and interacting factors in several biotic elements (fish, macroinvertebrates, phytobenthos and macrophytes). For that, a case study in the Sorraia basin (Central Portugal), a Mediterranean system mainly facing water scarcity and diffuse pollution problems, was chosen. To develop the proposed framework, a combination of process-based modelling to simulate hydrological and nutrient enrichment stressors and empirical modelling to relate these stressors - along with land use and natural background - with biotic indicators, was applied. Biotic indicators based on ecological quality ratios from WFD biomonitoring data were used as response variables. Temperature, river slope, % of agriculture in the upstream catchment and total N were the variables more frequently ranked as the most relevant. Both the two significant interactions found between single hydrological and nutrient enrichment stressors indicated antagonistic effects. This study demonstrates the potentialities of coupling process-based modelling with empirical modelling within a single framework, allowing relationships among different ecosystem states to be hierarchized, interpreted and predicted at multiple spatial and temporal scales. It also demonstrates how isolated and interacting stressors can have a different impact on biotic quality. When performing conservation or management plans, the stressor hierarchy should be considered as a way of prioritizing actions in a cost-effective perspective.

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1. Introduction

Riverine environments have been increasingly imperilled by human activities and have become one of the most degraded systems in the world (Sala et al., 2000; Gleick, 2003). Degradation of rivers is caused by a multitude of individual stressors, originating from drivers such as agriculture, urbanization and climate change, which affect ecological patterns and processes through a highly and increasingly intricate cause-effect chain (Hering et al., 2015; Gieswein et al., 2017). The implementation of effective river management actions and appropriate ecological restoration actions greatly relies on the ability of researchers to disentangle this complex cause-effect chain into simple models that are capable of providing guidance for managers (Hering et al., 2015). For example, modelling frameworks that project multiple stressor effects on biological components of ecosystems under scenarios of changes in drivers and measures may provide especially useful tools to support decision making. Although there are several examples of such attempts (e.g. Fernandes et al., 2016; Segurado et al., 2016), these are still major challenges that river ecologists and managers are currently facing.

Rivers, because of their particular nature, pose additional challenges to assess and model the effects of multiple stressors. Multiple stressor combinations vary deeply along river longitudinal gradients and among different ecoregions (Schinegger et al., 2012), causing difficulties in disentangling their effects on biotic components from natural causes because of the co-variability of environmental conditions (Alahuhta and Aroviita, 2016). Moreover, very often the effect of single stressors may depend on the environmental and biotic settings where they are acting. Several studies show biotic alterations associated with human-induced disturbances (Branco et al., 2013) that have a strong regional pattern in terms of the degree of impact imposed on streams. Another challenge posed by rivers comes from their particular network structure. Rivers have a directionality imposed by flow but they are more than “ribbons of aquatic habitat” (Fausch et al., 2002) because they form hierarchical dendritic network structures (Cote et al., 2010). These hierarchical, dendritic, directional networks are heterogeneous and continuous, with longitudinal, lateral, vertical, temporal (Ward, 1989) gradients that change at different scales (Frissell et al., 1986) and regions (Hering et al., 2015). This complexity severely hampers the ability to implement effective management actions in a river basin, especially if the goal is to achieve holistic targets e.g., taking into account all biotic quality elements and not do an over-“ribbon-like”-simplification.

The Water Framework Directive (WFD - European Commission, 2000) enforced the use of several biotic elements as indicators of surface water quality as an alternative to just water quality (Moss, 2007). The WFD involves defining biotic indicators of specific stresses, and their aggregation in the so-called one-out-all-out principle, but does not necessarily reflect a reliable indication of multiple stressors that recognize an integrated assessment of ecosystem health and mal-functioning (Hering et al., 2010). Additionally, most studies analyse solely the effect of individual stressors - a change in the environment that forces a response by the biological group of interest (Underwood, 1989) – on biotic indicators (Birk et al., 2012), notwithstanding the fact that often the response of an indicator to an isolated stressor is “wedge-shaped” – a clue that there are additional pressures at work that are expressed when the intensity of the isolated studied stressor is relatively low (Thomson et al., 1996; Friberg, 2010). It seems thus apparent that stressors interact, and, by doing so, create complex non-linear impacts. River systems are chiefly altered by hydromorphological degradation and diffuse pollution (EEA, 2012), which are themselves composed of several individual components. River regulation is widespread and severely alters flow velocity and water depth, creates vertical outflow drops that modify thermal and hydrology regimes of river systems and promotes the loss of original habitat which reduces heterogeneity and hampers the movement of river species (Segurado et al., 2013; Branco et al., 2014). Additionally, water quality is increasingly being deteriorated through urban, industrial and agricultural waste water. The combined impact of all these alterations has changed dramatically the constitution of river biotic communities (Allan, 2004).

Nowadays, increased water demand and climate change are likely to increase the magnitude and number of stressors acting upon river ecosystems and increase possible interactions. The interaction of different stressors can be manifold: additive when the response is predicted by the sum of the responses to isolated stresses; synergistic when the combined effect is greater than the sum of the effects of isolated stresses; or even antagonistic by creating responses smaller than those predicted (Underwood, 1989, but see Piggott et al., 2015 for an extensive review of the concepts). Deviations from additive effects among stressors tend to dominate, as shown by several studies (Côté et al., 2016; Nõges et al., 2016; Schinegger et al., 2016; Teichert et al., 2016; but see Gieswein et al., 2017 for opposing conclusions). Although studies focused on multiple stressors in aquatic environments are increasingly found in the literature (e.g. Ormerod et al., 2010; Côté et al., 2016; Feld et al., 2016; Jackson et al., 2016; Leal et al., 2016; Schinegger et al., 2016; Teichert et al., 2016), there is still a generalized lack of mechanistic understanding of stressors' interactive effects, which is a barrier for the prediction of responses to changing environments, risk assessment, management, impact mitigation and restoration of ecosystems (Vinebrooke et al., 2004). The use of models facilitates the prediction of management and conservation actions and by doing so facilitates cost-effective measures to be selected for future application. But, models are just a simplification of reality. This is more evident for models applied to river networks given their intrinsic complexity. Although there are large numbers of unforeseeable eventualities, the use of models in river systems is accepted as a standard practice with relevant knowledge arising from them (Feld et al., 2016).

The main goal of this work is to understand the interplay between the effects of multiple stressors, land use, reach scale attributes and climate on several biotic quality indicators in the Sorraia Basin, a typical Mediterranean basin located in SW Portugal. The Sorraia River is mainly affected by water scarcity - both as a consequence of its Mediterranean nature and an extensive water abstraction for irrigation - and nutrient enrichment from diffuse pollution from agriculture. This case study is part of one of the modelling framework approaches developed within the MARS project (Managing Aquatic Ecosystems and Water Resources Under Multiple Stress; Hering et al., 2015; Feld et al., 2016) that aims to predict effects of multiple stressors at the basin scale under different future climate change models, storylines and management scenarios. For this purpose, a process-based approach is used to estimate several stressors related to the hydrological regime and nutrient loads which is then coupled with an empirical modelling framework to calibrate models relating these stressors and other sources of variability with four common WFD biotic quality elements: fish, macroinvertebrates, macrophytes and phytobenthos. This work specifically looks at the stressors and gradients at play in this basin, identifies the stressor hierarchy and tests interactions among stressors in their effects on the biotic indicators. By doing so, this work, besides highlighting some specificities of working under a multi-stressor framework towards managing entire river basins that will predictably be affected by future alterations, advances knowledge and provides a theoretical basis that will facilitate management and conservation planning.

2. Materials and methods

2.1. Study area

The case study focused on the Sorraia Basin (Fig. 1), which has an area of 7730 km^2 and a length of 155 km. It flows towards the Tagus River estuary (outlet - latitude 38.83 and longitude −8.99) and is the Tagus tributary with the largest basin area.

The Sorraia Basin is characterized by a Mediterranean climate with an average annual air temperature of 15.2 °C that ranges from 21,6 °C

in the summer to 9.4 in the winter. The average annual precipitation is about 600 mm, from 400 mm in dry years to up to 900 mm in wet years. The average monthly precipitation is 50 mm, ranging from 25 mm in summer months to 70 mm in winter months. Approximately 41% of the Sorraia's basin area is forest, 28% range-grasses, 17% agriculture, 9% pine, 2% orchard, 2% urban and industrial and 1% pasture (Mateus et al., 2009). The two reservoirs in the basin affect runoff at the gauging stations. Natural flow is substantially reduced by water abstraction for irrigation. The Sorraia Basin has a total of 153,099 inhabitants with a density of 20 hab/km^2, mainly concentrated in three core areas: Ponte de Sôr (16,722 inhabitants), Samora Correia (17,123 inhabitants) and Coruche (19,944 inhabitants) (INE, 2012).

According to the Tagus River Basin Management Plan (APA, 2012), the main pressures on the basin are: (1) hydromorphological changes, (2) diffuse pollution, (3) municipal discharges, (4) flow regulation and (5) extraction of water. Key ecosystem services identified by the RBMP are: (1) water for irrigation, (2) recreation services and (3) waste water treatment. The ecological status of 122 water bodies, in which the biotic component was based on the four biotic quality elements (phytobenthos, macrophytes, macroinvertebrates and fish) considered in the present work, is: 54 good (44%), 15 moderate (12%), 12 poor (10%), 2 bad (2%) and 39 (32%) unclassified. The main causes of poor or failing status in the basin are mainly related to the water demand for agricultural purposes, which in the Sorraia basin is the highest within the Tagus River Basin (26% of total need). Nutrient loads from agriculture, livestock and urban origin, mainly in the alluvial valley, are also important potential causes of poor status in the basin.

2.2. Process based modelling - deriving stressor variables

To simulate daily variation of stressors related to hydrological processes and nutrient loads, the Soil and Water Assessment Tool (SWAT; Neitsch et al., 2005) model was used through its ArcSWAT interface for ArcGIS (ESRI, Redlands, CA, USA). SWAT is a process-based semi-distributed watershed model focused on land management at the reach or basin scale. It has growth parameters for about 100 plant species with crop interest and a vegetation growth model developed by the Grassland Laboratory of the USDA (United States Department of Agriculture). Topologically, SWAT divides the basin into subareas that are assumed to be homogeneous in their hydrologic response units (HRU) and infiltration or groundwater flow is computed based on empiric or semi-empirical formulations (as the SCS rainfall-runoff curves or soil-shallow aquifer-river transfer times). The hydrology of the model is based on the water balance equation, which includes runoff, precipitation, evaporation, infiltration and lateral flow in the soil profile.

The calibration procedure entails adjustments to the model parameters to obtain the best possible adherence of the modelled data to the measured data. To a priori determine which parameters should be adjusted in the model, flows modelled and observed in the same location and during the same period are compared and deviations interpreted. Model results were compared with data available from two monitoring stations from the Sorraia Basin: Moinho Novo and Ponte Vila Formosa (SNIRH; http://snirh.apambiente.pt/; accessed 30 July 2017). The period considered for the calibration and validation analyses was between 1996 and 2015. The coefficient of determination between the monthly mean flow modelled and observed was $R^2 = 0.69$ for Moinho Novo and $R^2 = 0.32$ for Ponte Vila Formosa; bias was -0.56 for Moinho Novo and 0.24 for Ponte Vila Formosa; the Nash-Sutcliffe efficiency (NSE) coefficient was 0.68 for Moinho Novo and 0.02 for Ponte Vila Formosa. For Total N, only the Moinho Novo had sufficient time series of data available for a proper estimation of model performance. For the mean annual mean of this parameter, the coefficient of determination was $R^2 = 0.59$, bias was 0.22 and NSE was -0.98.

Available GIS maps of topography, land use, soil type and climate, in the study area were used as inputs to the SWAT model. Topography was derived from the Shuttle Radar Topography Mission, with 90-m resolution (Jarvis et al., 2008). Soil physical properties were derived from the Portuguese Soil maps and Land use Capacity (http://www.dgadr.pt/cartografia; accessed 30 July 2017). Land use classification, adapted to the SWAT classification, was derived from the GSE Land M2.1 (Mateus et al., 2009), with 20 and 300-m resolution. Climatic maps, including daily or hourly precipitation, temperature, relative humidity and wind speed were derived from SNIRH (http://snirh.apambiente.pt/; accessed 30 July 2017).

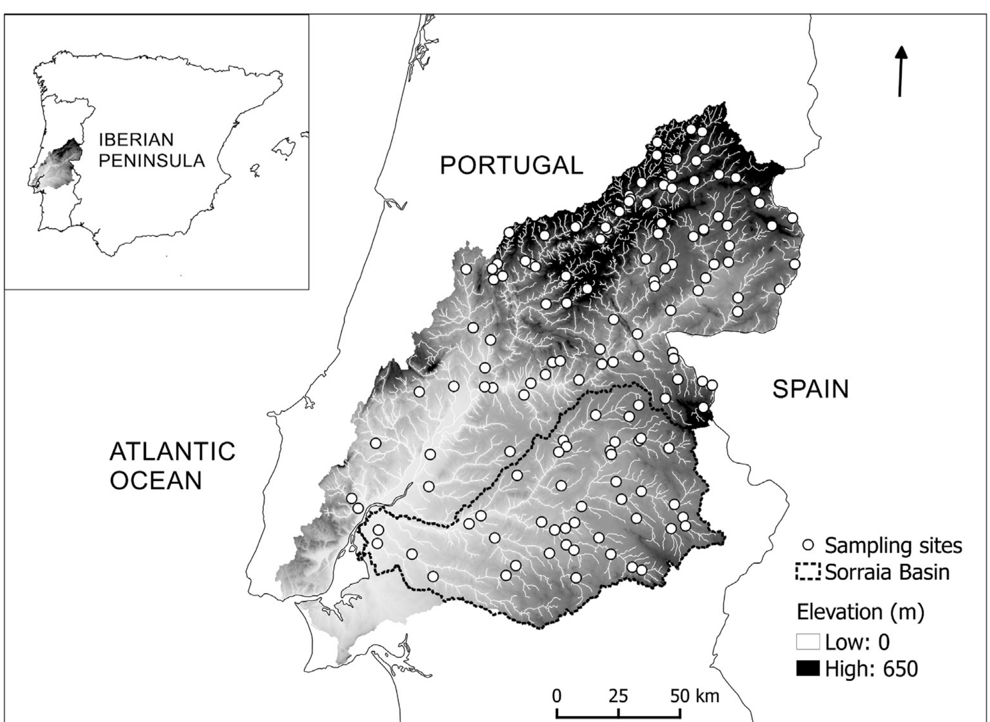

Fig. 1. River Tagus Basin, with a highlight of the study area (Sorraia Basin) and the location of sampling sites.

2.3. Empirical modelling - linking stressors to biotic indicators

To encompass a wider environmental and stressor gradient, we used data from the whole Tagus River Basin, where the Sorraia Basin is included, to fit empirical models relating biotic quality indicators, derived from biological monitoring data, with stressors. The dataset comprised 141 sites (Fig. 1) from the WFD biomonitoring program (Portuguese Environmental Agency, APA), with two sampling occasions (2010–11). Site selection included a set of least-disturbed sites used as reference sites. Remaining sites were selected to cover, as much as possible, different river types and the whole gradient of global disturbance measured in ordinal categories and based on hydromorphological alteration, water quality degradation and connectivity disruption.

The dataset included information on national biotic quality indices for four biotic quality elements: fish, macroinvertebrates, macrophytes and phytobenthos. For phytobenthos the national biotic quality index is the diatom metrics IPS (Indice de Polluosensibilité Sécifique) (Cemagref, 1982; Almeida et al., 2014). This index takes into account individual counts and species richness of all diatom taxon and it is an indicator of eutrophication, organic matter, acidification and salinity (Almeida et al., 2014). The national biotic quality index for macrophytes is the IBMR (Macrophyte Biological Index for Rivers), which is based on species abundance, ecological amplitude and trophic indicator value (Haury et al., 2006; Aguiar et al., 2014). It is considered a good indicator of nutrient inputs and/or heavy organic pollution (Haury et al., 2006). The macroinvertebrate national biotic quality index is the IPtI (Rivers Biological Quality Assessment Method — Benthic Invertebrates) (Ferreira et al., 2008; Feio et al., 2014). This index is based on the following metrics: number of taxa, species evenness, number of EPT (Ephemeroptera, Plecoptera, Trichoptera) families, evenness, IASPT (Iberian Average Score per Taxon index = IBMWP / number of families), log (selected ETD + 1) or EPTCD (log abundance of selected families of Ephemeroptera, Plecoptera, Trichoptera, Diptera, Coleoptera) (Feio et al., 2014). The index was developed using reference conditions based on land use, riparian condition, sediment load, hydrological regime, acidification and toxicity, morphological condition, nutrient enrichment and river continuity (Feio et al., 2014). The national biotic quality index for fish fauna is the F-IBIP (Fish-based Index of Biotic Integrity for Portuguese Wadeable Streams) (INAG and AFN, 2012; Segurado et al., 2014). The F-IBIP is a multimetric index based on parameters derived from fish assemblage composition and ecological functional groups (guilds) which differ among six fish-based river types. The index is based on twelve metrics scored separately by fish-type: number of native species, number of intolerant and intermediate species, % alien individuals, % intolerant individuals, % intolerant and intermediate individuals, % intolerant and intermediate Cyprinid species, % omnivorous individuals, % invertivorous individuals (excluding tolerant species), % potamodromous individuals, % reproductive generalist and "non-spawner" individuals, % lithophilic individuals and % water column individuals. The index was shown to be mainly responsive to water abstraction, presence of dams, presence of weirs, toxic risk and water quality (Segurado et al., 2014). The biotic quality indices were transformed into an ecological quality ratio (EQR) computed as the ratio between the original value of biotic quality index for a site and the value for reference or least disturbed sites of the same typology. An EQR close to zero indicates a site with a biological community that strongly deviates from those found in reference conditions.

The sampling protocol for phytobenthos followed European standard methods (CEN, 2003a). Most samples were collected in spring/summer. At least 5 pebbles covering in total at least 100 cm^2 of colonized surface were sampled per site. Diatoms were used as proxies for phytobenthos and counting of the cells followed standard procedures (CEN, 2004), with a minimum of 400 valves identified and counted. Macrophytes were sampled according to the European standards EN14184:2003 (CEN, 2003b) and EN14996:2006 (CEN, 2006). One-shot surveys per site were performed in spring–summer season (April to September). The sampling of macroinvertebrates followed the standard protocol established by Instituto da Água for the implementation of the Water Framework Directive in Portugal (INAG, 2008). A 50 m reach representing habitat diversity was defined for each site. Macroinvertebrates were sampled with a hand-net (0.25 m opening and 500 nm mesh size), each sample comprising six composite collections. Identification was performed mainly at the genus level. Fish sampling was performed by electrofishing following standard procedures (CEN, 2003c) for assessing fish species composition and abundance. Each site was sampled during spring–summer base flow. The fishing team progressed upstream in a zigzag pattern with single passes covering all present habitats (riffles, pools). Minimum sampled length was 20 times the mean wetted width of the channel.

Fifteen predictor variables were selected, including four land use pressure variables, two nutrient stressors, four hydrological stressors and five variables describing natural environmental variability (Table 1). Environmental variables were compiled from the CCM2 river network database (Vogt et al., 2007) for all river segments (river stretch between confluences). Land use pressures were derived from the CORINE landcover database (European Environmental Agency, 2010) as the percentage of area derived from a wide spatial scale corresponding to the whole upstream catchment. These pressure variables were used as a proxy of different environmental stressors (e.g. nutrient enrichment, water abstraction, sediment pollution, damming, flow regulation) rather than a stressor in itself. We considered it important to include these variables as predictors to control for the effects of other sources of variability that were not measured or modelled. The four land use variables were selected based on their potential effects on rivers. Agricultural land may be considered essentially a proxy for many different kinds of diffuse pollution in the form of nutrients (e.g. fertilizers, organic wastes from livestock activities) and toxic substances (e.g. pesticides). In addition, irrigation crops are a proxy of several hydrological alterations (e.g. water abstraction, patterns of extreme flow events). Urban areas are mainly a proxy for different types of point source pollution (e.g. from domestic and industrial wastes). Forests are essentially a proxy of several processes that contribute to reduce sediment in rivers and filter water pollutants. The percentage of area in the upstream catchment was computed with the RivTool software v1.0.0.1 (Duarte et al., 2016).

Table 1
List of candidate predictor variables. VIF – Variation Inflation Value with a threshold value of 3.

Predictor variables	Units	Range	VIF selection
Land use pressures			
Agriculture in the upstream catchment	%	0–96	Yes
Irrigated crop in the upstream catchment	%	0–19	Yes
Forest in the upstream catchment	%	0–83	Yes
Urban in the upstream catchment	%	0–12	Yes
Nutrient stressors			
Total phosphorus annual mean	mg/l	0.00–1.46	No
Total nitrogen annual mean	mg/l	0.95.69	Yes
Hydrological stressors			
Mean annual Flow	m3/s	0.13–129.01	Yes
Low flow pulse – number of events	.	0–40	Yes
Low flow pulse – mean duration (days)	Number of days	0.00–106.00	Yes
Mean annual flow alteration	%	0–35.56	Yes
Natural environmental variability			
Distance from source	km	2–981	No
River slope	%	0.01–75.01	Yes
Size of the upstream catchment	km^3	8–67,051	Yes
Mean annual temperature	°C	9.9–17.2	Yes
Mean total annual precipitation	mm	628–1552	No

The nutrient-related variables included two commonly used indicators of nutrient stress: total nitrogen and total phosphorus. Both stressors are main causes of eutrophication effects such as phytoplankton blooms and accelerated plant growth which results in low dissolved oxygen. The selected hydrological stressor indicators - mean annual flow, mean annual number of low flow events, mean annual duration of low flow events and mean annual flow alteration - are four uncorrelated measures of water scarcity. Low pulses were defined as periods during which the daily mean flow falls below the 10th percentile of the mean annual flow.

Because biotic indicators are affected by natural environmental gradients, it is crucial to control this effect when testing relationships with stressor variables. For the Tagus Basin we considered two main natural environmental gradients as the most relevant: a climatic gradient and a river longitudinal gradient. These gradients, expressed in our datasets by five environmental variables (Table 1), were included as candidate predictors in the empirical modelling framework to control as much as possible for the effect of natural background.

We found skewness problems in almost all predictors and therefore all explanatory variables were transformed using Box-Cox transformation (Box and Cox, 1964) followed by variable centering (mean $= 0$) and standardization (SD $= 1$), to express regression coefficients as standardized effect sizes. Collinearity among predictor variables was assessed through the use of VIFs (Variation Inflation Value) with a threshold value of 3 (Zuur et al., 2010). We used this criterion to exclude predictors in a stepwise fashion, by starting to remove the predictor with the highest VIF and repeating the VIF computation until all variable's VIFs were <3. We used the function vifstep of the package usdm that automatically performs the stepwise deletion based on a VIF threshold defined by the user (Naimi, 2015). Twelve variables were retained as candidates for inclusion in models (Table 1).

We ran several alternative empirical approaches following the overall procedure proposed by Feld et al. (2016) to analyse the impacts of multiple stressors in aquatic biomonitoring data. We performed a first exploratory analysis by running two machine learning techniques, Boosted Regression Trees (BRT; Elith et al., 2008) and Random Forests (RF; Breiman, 2001). After selecting the most relevant predictor variable candidates from the previous analyses, we quantified and tested both individual and multiple stressor effects through Linear Mixed Models (LMM; Zuur et al., 2009) using site as a random effect. Model selection was based on a multi-model inference procedure (Grueber et al., 2011) using the Akaike weight as a measure of the probability of the same model to be selected as the best approximating model using an independent dataset (Burnham and Anderson, 2002). We ran all combinations of models with no more than six predictor variables, to avoid selecting overly complex models. Pairwise interaction terms between hydrological and nutrient stressors were also included as candidate variables in the model selection procedure. Finally, the best approximating model, i.e. with the highest Akaike weight, was selected. LMM was also used to test pairwise interactions, i.e., to test significant deviations from additive effects and identify the interaction type (synergistic, antagonistic and opposing) based on the direction and intensity of such deviations. We included a year variable in all models to control for annual variability. In LMM we included year as a fixed factor because the number of classes (2 years) was not sufficient to use the variable as a random factor.

To allow comparisons of the goodness-of-fit among the three modelling techniques, we computed the correlations between observed and fitted EQR values for each biotic quality element. We also computed correlations between observed and predicted values using validation data. The validation procedure varied among the modelling technique. For BRT, the correlations were computed as the mean correlation of a 10-fold cross-validation procedure (see e.g. Elith et al., 2008 for further details). For RT, average predictions of “out-of-bag” samples used in each tree development were used to compute the correlations with observed EQR values. For LMM, we used a jackknife-based, or leave-one-out, cross-validation procedure to compute correlations of validation predictions with the observed response.

We used the three modelling approaches to rank the relative importance of stressors, land use, climate variables and stream attributes for each biotic indicator. In BRT, the importance of each variable in the model was estimated by averaging the number of times each variable was selected for splitting a tree as well as the squared improvement resulting from these splits (Friedman, 2001). The importance in RF models was based on Breiman-Cutler permutations in which, for each tree, the prediction errors are computed for “out-of-bag” data using both original and randomly permuted cases. Variable importance is then defined as the difference between the error rate of the original and permutated data averaged over all trees in the forest (Ishwaran, 2007). Because in this case low values of importance may take a negative sign, all negative values were truncated to zero. The relative importance of predictor variables in LMM was assessed based on the probability of each variable to be included in the best approximating models, estimated by summing the Akaike weights of all candidate models where the variable was included (Burnham and Anderson, 2002). For comparison purposes, values of importance were transformed in percentages in relation to the sum of importance values. The variables were then ranked for each modelling technique and biotic quality element and a mean rank was computed for each variable. Inconsistency among models in the rank of importance was then computed as the percentage of the difference between maximum and minimum position in the rank in relation to the maximum possible difference (equal to total number of variables). By checking the consistency across modelling techniques of the importance of predictor variables we assessed the uncertainty of predictions driven by the choice of the modelling technique.

All the analyses were performed with R version 3.3.2 (R Core Team, 2017) using packages gbm (Ridgeway, 2007) and dismo version 1.1-4 (Elith et al., 2008; Hijmans et al., 2013) for running BRT, randomForestSRC (Ishwaran and Kogalur, 2017) for running RF, lmerTest (Kuznetsova et al., 2014) and lme4 (Bates et al., 2015) to run LMM and MuMIN (Bartoń, 2016) to perform multimodel inference. We used R codes similar to those provided by Feld et al. (2016).

3. Results

3.1. Performance of empirical models

The empirical models relating the EQR of each biotic quality element with environmental, land use and stressor variables showed an overall good adjustment to the data (Fig. 2). Models fitted with LMM tended to show the best explanatory power. With BRT and RF, especially for phytobenthos, macrophytes and macroinvertebrates, fitted values tended to be biased towards an overestimation of EQR, especially for lower response values. These tendencies are confirmed by the correlations between fitted and observed EQR values (Fig. 3). Correlations using training data ranged from 0.81 for macrophytes using BRT and 0.99 for Fish using LMM. Correlations using validation data were lower, ranging from 0.27 for macrophytes using R and 0.81 for Fish using LMM. These validation correlations showed that models fitted with LMM also tended to show the best predicted power for the four biotic quality elements.

3.2. Relative importance of variables

Among all four biotic quality elements and modelling techniques, the variables most frequently ranked in the first three positions of relative importance (Tables 2 to 5) were % of agriculture (for phytobenthos, macroinvertebrates and fish), and the annual mean temperature (for macroinvertebrates and fish). The variables most frequently ranked in the last three positions of relative importance were Flow alteration (all biotic quality elements) and Number of low flow events

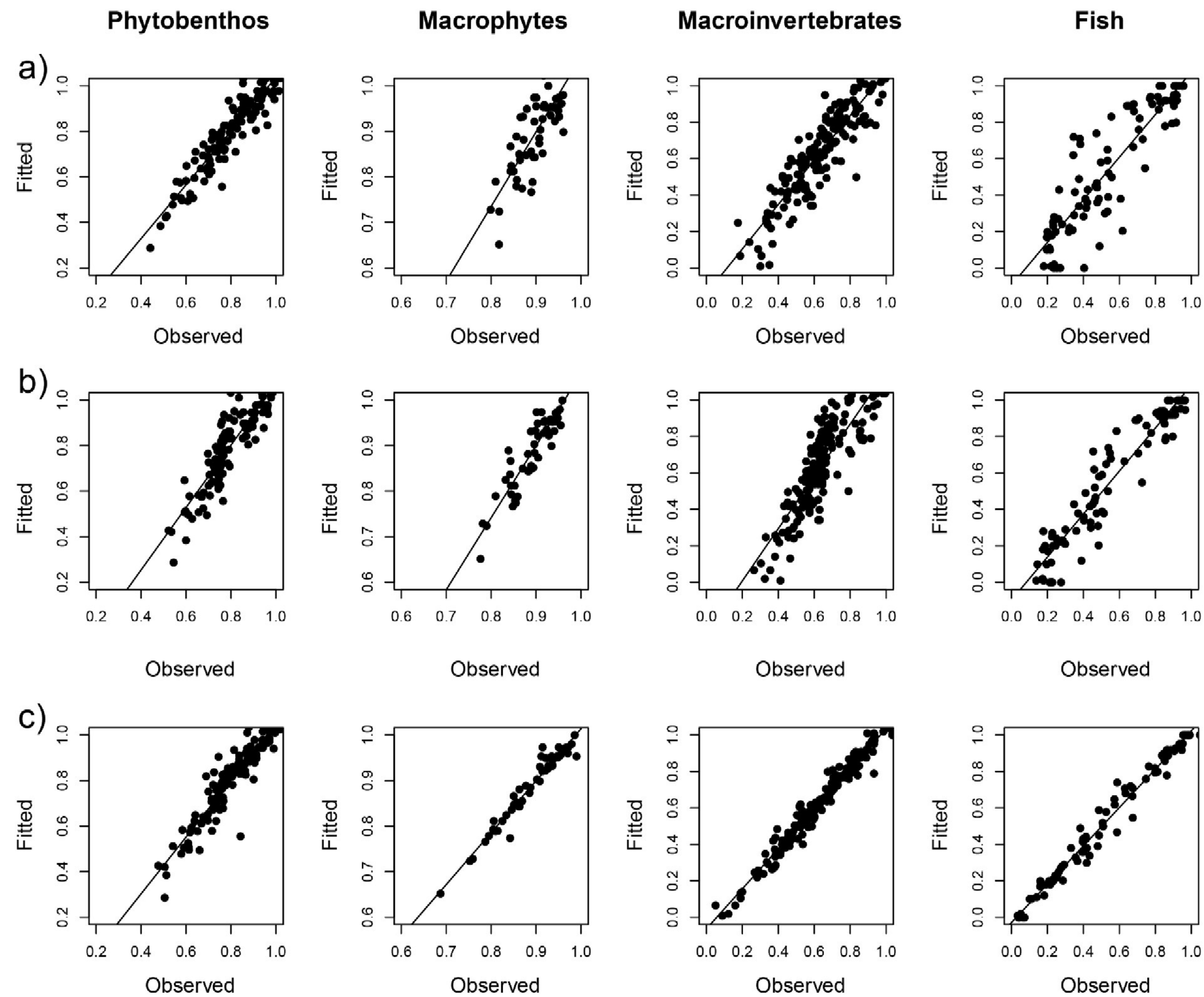

Fig. 2. Fitted versus observed values for each model and biotic quality element. a) Boosted Regression Tree; b) Random Forests; c) Linear Mixed Models.

(phytobenthos and macroinvertebrates). The variable year was the variable that was ranked more inconsistently among modelling techniques. Partial responses of each biotic indicator in each model are shown in Supplementary data (Appendix Aces).

For phytobenthos EQR (Table 2), two land use variables, % agriculture and % urban, were consistently the most important predictor variables among the three models. A stressor variable related to water scarcity (mean duration of low flow events) was ranked in the third position, followed by river slope and mean annual temperature. The % forest, number of low flow events and flow alteration were consistently the least important variables. Year was the variable that showed the most inconsistent rank of importance among the three models, ranked in the fourth position in LMM and in the 2 last positions in BRT and RF models.

The most important predictor variables affecting macrophytes EQR (Table 3) were a river segment attribute (river slope), a land use variable (% forest, mostly cork-oak land) and a hydrological stressor (mean annual flow), although inconsistently ranked in first, second or third place among the three models. Mean annual temperature, % irrigated croplands and flow alteration were consistently ranked in the last three positions among the models. Again, year showed the most inconsistent rank of importance among the three models.

For macroinvertebrates EQR (Table 4), the predictor variables ranked in the first three positions were % agriculture, mean annual temperature and % of irrigated croplands. In this case, outputs from LMM showed an overall inconsistency with those from BRT and RF. For example, year was ranked in the first position in LMM but in the last three positions in BRT and RF models. Size of catchment, flow alteration and number of low flow events were ranked in the last three positions, although their ranking showed weak consistency among methods.

Mean annual temperature, total N and % agriculture were the variables that had the highest rank of importance for fish EQR (Table 5). Mean duration of low flow events was the second most important stressor, although showing the highest inconsistency among models, ranked in the third from the last position according to the BRT model. Mean annual flow, year and flow alteration were ranked in the last three positions, but their rank order showed weak consistency among methods.

3.3. Effects sizes and interactions

The variables included in the best approximating LMM for each biotic quality element are shown in Table 6. Mean annual temperature, river slope, % agriculture and total N were the most frequent selected variables, all included in two of the four models. The predictor variables selected in the best approximating model and their effect sizes are not necessarily consistent with the ranks found in Tables 2–5 because these ranks take into account a large number of models with different combinations of variables.

The variables included in the phytobenthos model were river slope with a positive effect, two land use variables, % agriculture and % urban, both with a negative effect, and year. Percent agriculture and the % urban showed the highest effect size.

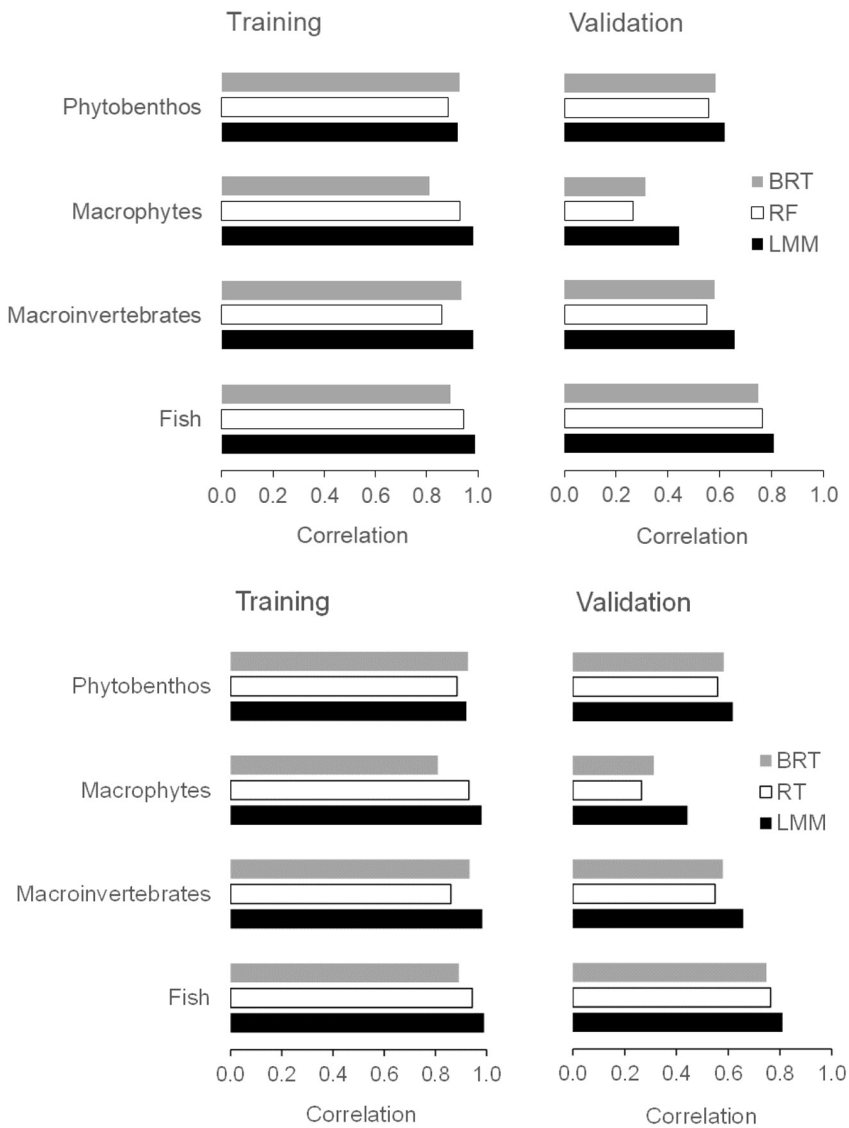

Fig. 3. Correlation between fitted and observed values for each model type and biotic quality element, based on the training dataset (left graph) and a validation procedure (right graph).

Table 2
Relative importance of variables as predictors of phytobenthos EQR in each model (BRT – Boosted Regression Trees; RF - Random Forests; LMM - Linear Mixed Models), mean rank of importance among models and inconsistency among models in the rank of importance (percentage of the difference between maximum and minimum position in the rank in relation to the total number of variables). Red indicates higher ranking and blue indicates lower ranking.

Variable	BRT	RF	LMM	Mean rank	Inconsistency (%)
% agriculture	23.13	35.91	17.35	1.00	0.00
% urban areas	15.61	19.60	16.99	2.00	0.00
Mean duration of low flow events	6.42	8.57	6.44	5.33	38.46
River slope	6.80	4.28	10.97	5.67	30.77
Mean annual temperature	7.29	3.57	6.13	6.00	30.77
Mean annual flow	10.49	5.49	4.64	6.00	46.15
Size of catchment	7.25	6.35	4.65	6.33	23.08
% irrigated croplands	7.26	3.55	5.84	7.00	30.77
Total N	5.59	7.44	4.53	7.67	46.15
Year	1.49	0.00	9.38	9.67	69.23
% forest	4.39	3.17	4.47	10.33	7.69
Number of low flow events	4.27	1.91	4.35	11.33	7.69
Flow alteration	0.00	0.15	4.27	12.67	7.69
Mean inconsistency (%)					26.04

Table 3
Relative importance of variables as predictors of macrophytes EQR in each model (BRT – Boosted Regression Trees; RF - Random Forests; LMM - Linear Mixed Models), mean rank of importance among models and inconsistency among models in the rank of importance (percentage of the difference between maximum and minimum position in the rank in relation to the total number of variables). Red indicates higher ranking and blue indicates lower ranking.

Variable	BRT	RF	LMM	Mean rank	Inconsistency (%)
River slope	17.80	22.95	15.56	2.33	15.38
% forest	18.76	31.63	8.06	2.67	30.77
Mean annual flow	24.05	30.12	8.02	3.00	38.46
% urban areas	15.82	10.08	7.39	5.00	23.08
Number of low flow events	4.66	0.16	11.45	6.33	61.54
Total N	5.51	0.51	6.52	6.67	23.08
% agriculture	2.41	2.49	5.18	7.33	30.77
Mean duration of low flow events	5.22	1.17	5.09	7.33	30.77
Size of catchment	1.77	0.43	8.95	7.67	46.15
Year	0.53	0.46	10.28	7.67	69.23
Mean annual temperature	1.99	0.00	4.46	10.67	23.08
% irrigated croplands	1.48	0.00	4.78	11.67	15.38
Flow alteration	0.00	0.00	4.26	12.67	7.69

The macrophyte model included also river slope with a positive effect, two stressor variables, total N and number of low flow events, both with a negative effect, and year. A significant interaction between total N and number of low flow events was found. The positive sign of the interaction regression coefficient, which goes in the opposite direction of the individual effects, indicates an antagonistic interaction between the two stressors, i.e., one stressor attenuates the effect of the other. This is confirmed by the 2D plot representing the co-effect of the two stressors on macrophytes EQR (Fig. 4). This plot also shows an opposing interaction, i.e., when one of the stressors is above a certain level, the effect of the other is inversed. However, most cases are located in the third quadrant of the plot, which indicates that an antagonistic interaction dominates.

The variables included in the best approximating model for macroinvertebrates were mean annual temperature, with a negative effect, two land use variables, % agriculture and % irrigated cropland, both with a negative effect, flow alteration, with a positive effect, and year. Percent agriculture and year showed the highest effect size.

The fish model included also mean annual temperature, with a positive effect, and two stressor variables, total N and mean duration of low flow events, both with a negative effect. A significant interaction between total N and mean duration of low flow events was found. Similarly to the macrophytes model, the positive sign of the interaction regression coefficient, with an opposite sign of the individual effects, indicates an antagonistic interaction between the two stressors. This is indicated by the 2D plot representing the co-effect of the two stressors on fish EQR (Fig. 4), showing that the colour change pattern along one variable axis changes along the other variable axis. This plot also shows an opposing interaction, although most cases are located in the third quadrant of the plot, which indicates a dominant antagonistic interaction.

4. Discussion

Managing such heterogeneous complex environments like river systems is a mammoth task that has to deal with a high degree of system-specificity and with mixed gradients of different nature (e.g. climatic, hydromorphological, biotic) that change the effect of a stressor along them. This translates into an impossibility of applying static measures with a homogeneous effectiveness throughout the stressor gradient, as the stressor itself changes its effect along other stressor gradients or even along environmental gradients. Scientists and managers should then understand how the response changes along these isolated or combined gradients, to adapt management actions to tackle stressors according to the specific gradient found in the basin of interest. The

Table 4
Relative importance of variables as predictors of macroinvertebrates EQR in each model (BRT – Boosted Regression Trees; RF - Random Forests; LMM - Linear Mixed Models), mean rank of importance among models and inconsistency among models in the rank of importance (percentage of the difference between maximum and minimum position in the rank in relation to the total number of variables). Red indicates higher ranking and blue indicates lower ranking.

Variable	BRT	RF	LMM	Mean rank	Inconsistency (%)
% agriculture	23.88	31.63	18.42	1.33	7.69
Mean annual temperature	9.69	9.39	9.11	3.00	15.38
% irrigated croplands	9.21	12.49	7.89	3.33	23.08
% urban areas	7.85	7.31	6.72	5.33	23.08
Total N	7.35	9.98	4.15	6.33	46.15
River slope	4.70	5.47	8.70	7.33	53.85
Year	6.18	2.68	18.74	7.33	76.92
Mean annual flow	7.83	4.77	3.67	8.67	53.85
% forest	7.50	4.58	3.99	8.67	30.77
Mean duration of low flow events	6.58	6.35	3.47	9.00	53.85
Size of catchment	6.55	5.00	3.96	9.33	23.08
Flow alteration	0.95	0.36	7.00	10.33	53.85
Number of low flow events	1.73	0.00	4.18	11.00	38.46
Mean inconsistency (%)					38.46

Table 5
Relative importance of variables as predictors of fish EQR in each model (BRT – Boosted Regression Trees; RF - Random Forests; LMM - Linear Mixed Models), mean rank of importance among models and inconsistency among models in the rank of importance (percentage of the difference between maximum and minimum position in the rank in relation to the total number of variables). Red indicates higher ranking and blue indicates lower ranking.

Variable	BRT	RF	LMM	Mean rank	Inconsistency (%)
Mean annual temperature	57.92	45.82	21.67	1.00	0.00
Total N	12.44	14.73	16.41	2.67	7.69
% agriculture	14.12	16.44	6.57	3.33	30.77
% forest	6.20	3.77	6.70	4.67	15.38
Mean duration of low flow events	0.26	4.64	8.41	6.00	61.54
River slope	2.14	3.02	5.15	7.00	15.38
% irrigated croplands	0.84	3.90	5.13	7.67	30.77
Number of low flow events	0.48	2.19	6.62	8.33	38.46
% urban areas	2.56	2.37	4.52	8.33	46.15
Size of catchment	1.33	2.57	4.36	9.67	38.46
Mean annual flow	1.63	0.54	4.42	10.00	38.46
Year	0.08	0.00	5.25	10.67	46.15
Flow alteration	0.00	0.00	4.79	11.67	23.08
Mean inconsistency (%)					30.18

work presented herein had the purpose of identifying difficulties and providing means to understand stressor importance and response variation along interacting stressor gradients.

4.1. Relative importance of variables

In the addressed case study there was a high impact of land use in the upstream drainage area on biotic indicators (see Liuzzo et al., 2015; Santos et al., 2015 and Sellami et al., 2016 for concurring previous findings). This was evident for all the studied biotic elements, and it is a consequence of the fact that land use variables tend to be a proxy for multiple stressors (Feld et al., 2016). For example, the presence of croplands is a proxy for very distinct single stressors, such as diffuse pollution, water abstraction and riparian habitat degradation that even may act synergistically. Additionally, both hydrologic and climatic variables were deemed important predictor variables, although for different biotic elements. This is understandable (Biggs et al., 2005), expected (Bonada and Resh, 2013; Gasith and Resh, 1999; Hershkovitz and Gasith, 2013; Wada et al., 2011) and previously demonstrated (Segurado et al., 2016). Water abstraction effects can severely affect lotic systems (Dewson et al., 2007; Wooster et al., 2016; Benejam et al., 2010; Lange et al., 2014), especially in Mediterranean regions and under the effect of climate and socioeconomic changes.

Albeit the aforementioned commonalities, all biotic elements varied in terms of the top ranking variables. Specifically, macrophytes were structured according to river slope, a variable that reflects the natural gradient between headwaters and lowland rivers – it was the only biotic element for which a “structural” variable ranked high in terms of importance. This is because of the ecology of this biotic element and the multiple effects of river slope – e.g. water velocity, sediment transport and residence time – that creates a very marked longitudinal variation in both the composition and structure of the aquatic and riparian vegetation communities (Manolaki and Papastergiadou, 2013). Fish, on the other hand, were the only element to rank a stressor (total N) among the most important variables. This is because total N is related to the surrounding land use and those are also closely linked to known changes in the fish assemblage following structural “along-the-river” alterations. An increase in nutrient concentration may lead, in high insolation areas, to the proliferation of submerged macrophytes and to consequent severe impacts on freshwater fish (Pusey and Arthington, 2003). Branco et al. (2016) also found that, using dissolved oxygen as a proxy in an experimental setup, the input of organic pollution and subsequent degradation seemed to affect fish activity levels. It is known that in some cases the biotic quality status indicators give clear and expectable responses to human induced disturbances, but for other indicators there are weak responses to human stressors, with the strongest responses related to natural environmental variability and spatial processes (Alahuhta and Aroviita, 2016).

Apart from pure biological factors, other causes related to methodological options may also contribute to the observed differences among biotic quality elements in their responses to predictor variables.

Table 6
Summary of the best approximating LMM model, including the standardized effect size (SES), the standard error of the estimate (SE), the degrees of freedom (df), the *t*-test value of the coefficient and its associated p-value.

Variable	SES	SE	df	*t*-Value	p-Value
Phytobenthos					
(Intercept)	−58.150	39.805	57.300	−1.461	0.150
River slope	0.029	0.016	87.320	1.883	0.063
% agriculture	−0.046	0.017	85.760	−2.712	0.008
% urban	−0.042	0.016	82.680	−2.562	0.012
Year	0.029	0.020	57.300	1.481	0.144
Macrophytes					
(Intercept)	−72.876	32.106	20.430	−2.270	0.034
River slope	0.037	0.014	45.370	2.744	0.009
Number of low flow events	−0.031	0.012	55.000	−2.559	0.013
Total N	−0.023	0.014	47.360	−1.692	0.097
Year	0.037	0.016	20.430	2.298	0.032
Number of low flow events × total N	0.027	0.015	41.750	1.768	0.084
Macroinvertebrates					
(Intercept)	−279.103	36.871	50.990	−7.570	<0.001
Mean annual temperature	−0.043	0.023	132.600	−1.885	0.062
% agriculture	−0.100	0.021	132.250	−4.726	<0.001
Flow alteration	0.035	0.019	131.810	1.853	0.066
% irrigated croplands	−0.039	0.022	130.940	−1.748	0.083
Year	0.139	0.018	50.990	7.587	0.000
Fish					
(Intercept)	0.560	0.028	79.960	20.283	<0.001
Mean annual temperature	−0.212	0.029	79.960	−7.306	<0.001
Mean duration of low flow events	−0.012	0.026	86.130	−0.438	0.662
Total N	−0.086	0.029	81.880	−2.928	0.004
Mean duration of low flow × total N	0.060	0.028	62.190	2.156	0.035

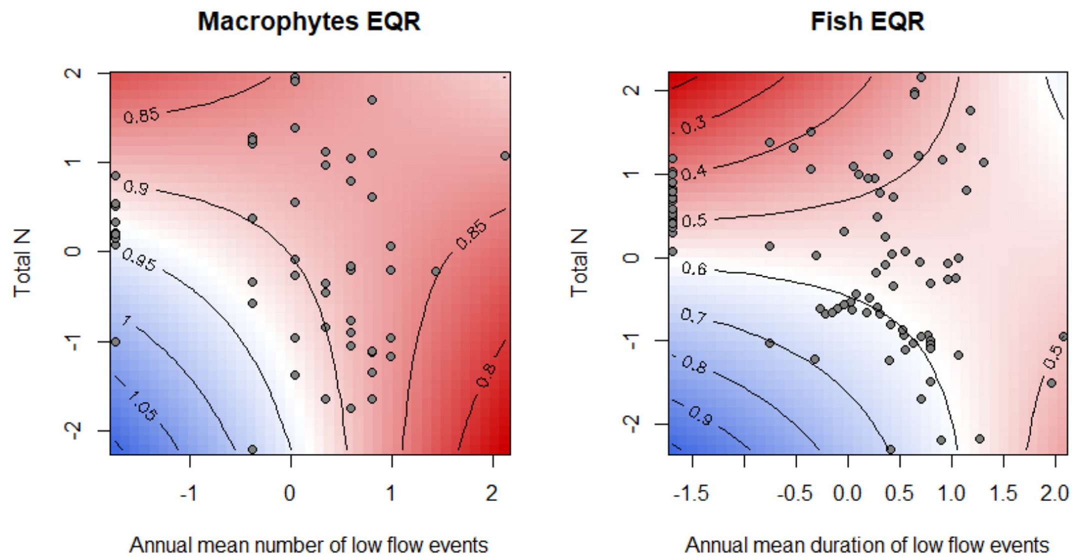

Fig. 4. Plots showing the pairwise interactions in the LMM model for macrophytes (left) and fish (right). Response variables are expressed by colour intensity, varying from low EQR values (red) to high EQR values (blue). Dots represent the true observations and may be used to check which portion of the plot is more supported by data.

Differences may arise because of distinct rates of assemblage change in the presence of stressors, i.e., they might be attributed to the temporal resolution at which predictor variables were compiled. Phytobenthos and macroinvertebrates, given their shorter life cycles in comparison to macrophytes and fish, are expected to respond more promptly to environmental change and hence to respond to shorter time windows. This might explain why selected LMM included more land use variables and fewer individual stressors in the case of phytobenthos and macroinvertebrates. The time window (annual mean) to derive individual stressors may not be the most adequate for these organisms. Responses may also be influenced by the spatial scale at which variables were compiled or simulated (e.g. HRU size in SWAT simulations), mainly because of different dispersal abilities among organisms. For example, fish are known to be more responsive to stressors acting at wider spatial scales than other organisms (Harris, 1995) because they can easily move to more favourable environments in face of local disturbances. Therefore, differences found among biotic quality elements in their response to predictor variables may have been partially driven from the use of common temporal and spatial resolutions in the modelling framework.

The influence of sampling protocols and site selection in model precision and accuracy, and hence in their generality and predictive power, cannot be discarded (Stevens & Olsen, 2004; Hughes & Peck, 2008; Hughes et al., 2000, 2012). Even scientifically informed sampling designs involved in biomonitoring program are necessarily constrained by funding resources (limiting the number of samples), logistics (e.g. site accessibility) and subjective human decisions (Hughes & Peck, 2008). Sampling decisions that originated data used in the present work are no exception and their effects on models are inevitable. On the other hand, the global disturbance gradient that considered for sampling site selection of the biomonitoring database might not totally reflect the gradient of stressors that were dealt with in this work. Additionally, because the case study basin is dominated by a Mediterranean landscape which has been shaped from centuries of human activities, there is an overall lack of minimally disturbed catchments (Segurado et al., 2011) which necessarily shortens stressor gradients, with implications on modelling results (Feld et al., 2016; Leitão et al., 2017). Despite all the potential methodological effects on the results of the several modelling approaches, a certain degree of confidence is ensured given that the biomonitoring data used in this work was originated from sampling protocols that strictly followed European standards. In addition, sampling protocols for the four biotic quality elements were all WFD compliant which allowed the biotic quality indices to be subjected to the WFD intercalibration process to harmonize quality class boundaries with other European indices (Aguiar et al., 2014; Almeida et al., 2014; Feio et al., 2014; Segurado et al., 2014).

4.2. Stressor interactions

The present paper also aimed at identifying and understanding stressor interactions at play in the Sorraia Basin. Significant stressor interactions (LMM) were found for two biotic elements, macrophytes and fish. In both cases the interactions were found to be opposing (see Feld et al., 2016). But, if there is a focus on the data supported portion of the stressors gradients, it becomes evident that, in fact, the stressor interaction taking place is mostly antagonistic (see Feld et al., 2016). So, the interaction along the full gradient of the two interacting stressors is opposing but it changes along the gradient. When looking at partial gradients the interaction may differ from opposing. This highlights the need to analyse the full gradient of the stressors (Branco et al., 2016; Schinegger et al., 2016). Furthermore, the resulting opposing interaction might be a mathematical artefact of the model in the portion of the gradient under-represented by data. This is most likely to be the case of the interactions detected in this study. In fact, the plots representing interactions in a model must be interpreted very carefully. Original data must always be projected in the plot to check if there are regions of the modelled relationship that are not well supported by the data.

A synergistic interaction between hydrological stressors and nutrients, rather than the observed antagonistic interaction, was expected. This is because water scarcity would expectedly amplify the effects of nutrient loads by decreasing the natural diluting property of rivers (Blasco et al., 2015). An important aspect to be considered in the particular case of pairwise interactions in the context of regression-based modelling, which is the typical approach when analysing biological monitoring data, is that significant deviations from additive effects occur when one variable affects the slope of the response to the second variable. This peculiarity is very distinct from interactions inferred from typical factorial designs of controlled experiments, which do not involve estimates of response rates, but usually simple comparisons between stressed and unstressed conditions. So, one possible explanation for antagonistic interactions among stressors is that when stressor 1, e.g. total

N, is acting alone, the slope of the increase of the effect along the stressor gradient is expectedly steeper because at low stressor intensity, the biotic quality is at its maximum (low values of both stressors), i.e., for a small increase of the stressor there is a more pronounced decrease in the biotic quality. When stressor 2, e.g. duration of low flow events, is acting the rate of the increase of the effect along the stressor 1 gradient might be weaker because even at low values of stressor 1, the biotic quality is already being affected by stressor 2. Additionally, it is expected that when a single stressor dominates, the biotic response may reach a level beyond which it will not decrease even in the presence of a second stressor. In fact, a recent meta-analysis found that antagonistic effects among stressors prevail in the literature focused on freshwater ecosystems (Jackson et al., 2016). The antagonistic effect found between hydrological and nutrient stressors does not necessarily mean that the presence of one stressor attenuates the effect of the other uniformly along the stressors' gradient (Brown et al., 2014). It is often claimed that efforts to mitigate stressors are least effective in systems where antagonistic interactions prevail (Brown et al., 2013; Piggott et al., 2015) but this is very contingent on the position of the data along stressor gradients, which determines whether antagonistic effect deviates more or less from the additive effect.

4.3. Implications for management

The results of this work clearly highlight the importance of having more than one biotic element as a management/conservation goal, or as an indicator for management/conservation prioritization, as each element responds very differently to the diverse categories of environmental variables. Additionally, it is important to consider several environmental variables of each category (e.g. hydrology, climate, land-use), as for some elements some variables of the same category can either rank high or low in terms of importance in explaining the registered variability, and their relative positions may drastically change between biotic elements. The overall image is important to fully ascertain a basin status and to define management/conservation practices as each biotic element is differently affected by the natural background and stressors. Even if a stressor is considered as an important variable for two biotic elements, their responses to stressor levels may be different as the subsidy-stress thresholds change (or not) between elements (Odum et al., 1979).

The high degree of inconsistency that was attained for the results of the three modelling techniques for all studied biotic elements shows that, although all the pursued techniques are adequate for the data and questions at hand, the impact of the choice of the modelling technique on the results is large. This is even more evident when focusing on the importance of the year that only ranked high, in terms of importance, for the LMM approach. The high degree of inconsistency between modelling techniques demonstrates that any management or conservation decisions that immerge from distinct modelling technique outputs may originate dramatically distinct results. This work clearly shows that such a basin-wide management endeavour, cannot be properly fulfilled looking to just one or two biotic elements and by conducting analysis based on a single technique. The approach to basin-wide management has to be done holistically.

The way how modelled stressor effects behave along other gradients suggest that scale effects must be taken into account when dealing with stressor gradients and interactions. If in a given basin a certain stressor only expresses a portion of its full gradient, only that portion should be considered if the goal is basin management. The scope of the analysis must be in line with the scale of the task ahead. If not, management and conservation decisions may be skewed and not fully effective because they were thought in a way to be effective in portions of the stressor gradient that are never expressed in that particular river basin. Some ecological and biological traits have been shown to disentangle the effects of interacting stressors. So, the response variables elected for analysing multiple stressors should be mechanistically relevant to the stressors (Townsend and Hildrew, 1994; Poff, 1997; Statzner and Beche, 2010; Doledec and Statzner, 2008). Additionally, when looking at several biotic elements with different dispersion abilities, the effect of spatial processes should be considered, although most often they are not reflected in the large spatial scale at which bioassessments are undertaken (Frimpong et al., 2005; Aroviita et al., 2009; Heino, 2013; Alahuhta et al., 2013). Even though standard field protocols have been proved to be able to be used across very large areas (Paulsen et al., 2008), it may be valuable to use concepts such as “extent” and “risk” (for further details see: Paulsen et al., 2008 and Sickle and Paulsen, 2008) that present stressor effects as relative magnitudes or importance across a region.

Because nutrient enrichment stressors and land uses associated with agriculture were shown to have a major overall impact on the target biotic indicators, a big effort should be focused on limiting nutrient loads into aquatic ecosystems in future river management plans of the case study basin. This may be accomplished for example by increasing the efficiency of fertilization practices. A future increase of extreme low flow events are expected in Mediterranean regions according to most global and regional circulation models (IPCC, 2001), with an expected negative impact on biotic quality of rivers. Agriculture may exacerbate this effect through water abstraction and therefore an effort centered on the implementation of more effective irrigation schemes is also recommended.

4.4. Concluding remarks

This work demonstrates the potentialities of coupling process-based modelling with empirical modelling within a single framework that, through model projections under hypothetic scenarios, may help decision making at the basin scale. This is accomplished without loss of spatial resolution because predictions of biotic state may be computed for all river segments in a freshwater system network – while taking into account the effect of the upstream drainage area to all segments, merging Allan (2004) and Fausch et al.'s (2002) view on “riverscapes”. Such an approach facilitates plans of measures to be tested under several climatic and socioeconomic future scenarios, ensuring a cost-effective efficient basket of measures to be deployed depending on future developments, but also to detect best-practices and measures to increase the system resilience to the perceived future changes – acting as a prophylactic against forthcoming threats by present stressors. It further highlights how stressor interaction is still a difficult problem to tackle and how not looking at the full gradient of the stressors while looking at an appropriate response might lead to erroneous conclusions (Branco et al., 2016) and then to disastrous management decisions. Whereas interacting stressors are extremely important, one should not focus management solely on dealing with them, as there are often strong effects rising from isolated stressors. The understanding of the importance of the stressor is paramount. One should look at the effect size, either isolated or interacting, and prioritize management actions according to it.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2017.12.201.

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